IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. 10/677,734	Customer No. 23379
Applicant: Gardner et al.	Confirmation No. 4912
Filed: Oct 01, 2003	Group Art Unit: 1652
Docket No. UTSD:1510-1	Examiner: Swope, Sheridan

Title: *Foreign PAS Ligands Regulate PAS Domain Function*

SUPPLEMENTAL REPLY BRIEF

Honorable Board of Patent Appeals and Interferences
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Honorable Board:

We reply to the Answer dated Sep 04, 2007, which other than listing additional references on p.3, is identical to the Answer dated Jul 16, 2007, to which we responded in our original Reply Brief. We maintain our appeal from the Examiner's Jan 05, 2007 final rejection of claim 21.

I. THE EXAMINER HAS NOT PROPERLY REJECTED CLAIM 21 UNDER 35USC112, FIRST PARAGRAPH (Enablement).

The claim is directed to a method of changing a functional surface binding specificity of a selected PAS domain by (a) introducing into the hydrophobic core of the PAS domain a foreign ligand of the PAS domain; and (b) detecting a resultant change in the functional surface binding specificity of the PAS domain, wherein …the binding specificity is an intramolecular binding affinity of the PAS domain, detected as a change in chemical shifts detected by 1H/15N-HSQC NMR.

As explained in the Specification, suitable foreign ligands may be screened from libraries of synthetic or natural compounds, and conventional SAR analyses provide ligands of higher affinity and/or specificity (Specification, p.6, lines 9-10). This process was specifically exemplified with HIF2a PAS B, wherein a library of 772 compounds (Specification p.13, lines 6-14) was screened for HIF2a PAS B binding using 1H/15N-HSQC NMR; as seen in Figure 1, 21 hits were obtained for HIF2a PAS B (see also, Specification, p.18, line 1). From these the inventors developed a "lead" HIF2a PAS B ligand (Specification, top of p.31).

The Specification confirms that the foreign ligands bind the hydrophobic core of HIF-2 PAS B, and as a result, alter the functional surface binding specificity of the PAS domain, wherein the binding specificity is an intramolecular binding affinity of the PAS domain detected as a change in chemical shifts detected by 1H/15N-HSQC NMR:

> Our structural studies confirm that core ligand binding in each HIF-2 PAS B and ARNT PAS B induces distal changes in PAS domain structure, and functional binding studies confirm resultant changes in DNA:HIF-2 :ARNT transcription complex formation and DNA binding specificity (e.g. Michel et al., Biochim Biophys Acta. 2002 Oct 11;1578(1-3):73-83). Table 3 show exemplary identified foreign ligands of HIF-2a PAS B and ANRT PAS B, respectively, which specifically bind within their hydrophobic cores and disrupt complex formation. Specification, p.18, lines 21-26.

The practitioner does not require any *a priori* structural characteristics of the recited "foreign ligand" to practice the method. As demonstrated, the method is typically practiced using a library of compounds which need not be structurally characterized.

As for the cited step of introducing into the foreign ligand into the hydrophobic core of the PAS domain, this can be effected by simply mixing a PAS domain-containing protein with the ligand in solution (Specification, p.20, lines 7-8).

As demonstrated by the Specification (supra) and the uncontroverted evidence of record (Expert Declaration under 37CFR1.132, attached below), one skilled in the art would have been readily able to practice this without undue experimentation.

We do not harbour any "belief" that our claim is limited to some "combined screening assay/use method" (Answer, p.10, first full para.); in fact, we have no idea what is even meant by that allegation. The claim speaks for itself. It recites a two-step (introducing and detecting

steps) method, that the Specification amply describes, enabling one skilled in the art to practice it without undue experimentation.

The Examiner's repeated reliance on some "lock and key" analogy is misplaced. This hundred-year old analogy is expressly premised on a falsehood; that proteins are rigid bodies comprising fixed ligand receptacles. It is particularly inapt here, where the PAS domain has no NMR-apparent a priori formed ligand cavity.

Turned on its head, the analogy highlights the non-obviousness of the invention: the inventors have shown that a lock with no apparent keyhole, and previously not known to even be an operative lock, can in fact swallow a variety of keys, wherein the swallowed keys induce various conformational changes to the lock that can be detected as changes in chemical shifts detected by 1H/15N-HSQC NMR.

II. THE EXAMINER HAS NOT PROPERLY REJECTED CLAIM 21 UNDER 35USC112, FIRST PARAGRAPH (Written Description).

The claim is directed to a method of changing a functional surface binding specificity of a selected PAS domain by (a) introducing into the hydrophobic core of the PAS domain a foreign ligand of the PAS domain; and (b) detecting a resultant change in the functional surface binding specificity of the PAS domain, wherein the PAS domain is HIF2a PAS B….

As explained in the Specification, suitable foreign ligands may be screened from libraries of synthetic or natural compounds, and conventional SAR analyses provide ligands of higher affinity and/or specificity (Specification, p.6, lines 9-10). This process was specifically exemplified with HIF2a PAS B, wherein a library of 772 compounds (Specification p.13, lines 6-14) was screened for HIF2a PAS B binding using 1H/15N-HSQC NMR; as seen in Figure 1, 21 hits were obtained for HIF2a PAS B (see also, Specification, p.18, line 1). From these the inventors developed a "lead" HIF2a PAS B ligand (Specification, top of p.31).

The Specification confirms that the foreign ligands bind the hydrophobic core of HIF-2 PAS B, and as a result, alter the functional surface binding specificity of the PAS domain, wherein the binding specificity is an intramolecular binding affinity of the PAS domain detected as a change in chemical shifts detected by 1H/15N-HSQC NMR:

> Our structural studies confirm that core ligand binding in each HIF-2 PAS B and ARNT PAS B induces distal changes in PAS domain structure, and functional binding studies confirm resultant changes in DNA:HIF-2 :ARNT transcription complex formation and DNA binding specificity (e.g. Michel et al., Biochim Biophys Acta. 2002 Oct 11;1578(1-3):73-83). Table 3 show exemplary identified foreign ligands of HIF-2a PAS B and ANRT PAS B, respectively, which specifically bind within their hydrophobic cores and disrupt complex formation. Specification, p.18, lines 21-26.

The practitioner does not require any *a priori* structural characteristics of the recited "foreign ligand" to practice the method. Structure/function information about select ligands (e.g. Answer, p.15, third full para.) is not relevant to possession of the method we claim. As demonstrated, the method is fully described, and typically practiced using a library of compounds which are not a priori structurally or functionally characterized.

As demonstrated by the Specification (supra) and the uncontroverted evidence of record (Expert Declaration under 37CFR1.132), the Specification amply describes and exemplifies the claimed methods to one skilled in the art.

Finally, we do not harbour any "belief" that our claim is limited to some "combined screening assay/use method" (Answer, p.15, last para.); in fact, we have no idea what is meant by that allegation. The claim speaks for itself. It recites a two-step (introducing and detecting steps) method, that the Specification amply conveys possession of, and in fact, repeatedly exemplifies.

III. THE EXAMINER HAS NOT PROPERLY REJECTED CLAIM 21 UNDER 35USC103(a) OVER Vogtherr (EXS. 2003, 93, 183-202) OR Amezcua (Structure 2002, 10, 1349-61) IN VIEW OF Ema (PNAS USA, 1997, 94, 4273-8) IN FURTHER VIEW OF Fukunaga (J Biol Chem 1995, 270, 29270-8).

The claim is directed to a method of changing a functional surface binding specificity of a selected PAS domain, wherein the PAS domain is folded in its native state, and comprises a hydrophobic core that has no NMR-apparent a priori formed ligand cavity by (a) introducing into the hydrophobic core of the PAS domain a foreign ligand of the PAS domain; and (b) detecting a resultant change in the functional surface binding specificity of the PAS domain, wherein the

PAS domain is HIF2a PAS B, and the binding specificity is an intramolecular binding affinity of the PAS domain detected as a change in chemical shifts detected by 1H/15N-HSQC NMR.

Vogtherr (2003) generally describes the use of NMR-based screening for lead discovery; Amezcua (2002) describes the used of NMR to detect ligand binding to PAS kinase; Ema (1997) reports that *HIF1a* heterodimerizes with Arnt (note that HIF1a is structurally and functionally distinct from the recited HIF2a; Sowter (Cancer Res, 2003, 63, 6130-34); and Fukunaga (1995) reports identification of functional domains of the aryl hydrocarbon receptor.

Prior to the present disclosure, HIF was known to be regulated in several ways by oxygen availability, but only via mechanisms that are based on oxygen-sensitive enzymes that covalently modify portions of the HIFa subunit at sites distant to the PAS domains (Expert Declaration under 37CFR1.132, attached below, and citations therein). These prior findings taught away from any expectation that the HIF PAS domains would be sensory. In addition, HIF2a PASB presents a well-folded domain lacking the dynamic regions of PASK PAS A (Amezucua et al., 2002, p.1352, col.1, lines 10-12) and long insertion loops of NPAS2 PAS A (Erbel et al., 2003, PNAS 100, 15504-9), further removing any expectation of core ligand binding. Furthermore, we have of record uncontroverted evidence in the form of an expert Declaration, confirming that one skilled in the art at the time of our filing would not have expected HIF2a PAS to provide a core for sensory ligand binding.

The Action correctly states that the inventors' prior publication (Amezucua et al., 2002) disclosed that hPASK PAS A has a well-packed hydrophobic core, yet was able to bind small organic molecules, and speculated that other PAS domains, including those that do not copurify with ligands when isolated from natural sources, *may* serve sensor roles in vivo. However, as noted above, hPASK PAS A also demonstrated "unusual flexibility … near the ligand binding sites" (Amezucua et al., 2002, p.1352, col.1, lines 10-12). This unusual flexibility near the ligand binding site is what led the authors to hypothesize that hPASK PAS A might be able to bind small organic molecules despite its NMR-apparent well-packed core (id.) -- and this unusual flexibility near the ligand binding site is not present in HIF2a PASB. Also as noted above, unlike the situation with hPASK, HIF was previously known to be regulated by non-PAS mechanisms. It is because of their structural and functional distinctions, that there is no

suggestion anywhere that HIF2a PAS would or could provide a receptor for small organic molecules, and no one skilled in the art would try to impose on HIF2a PASB an inference drawn from a functionally and structurally distinct protein like HPASK PAS A. And the foregoing is documented in the uncontroverted expert declaration of record.

Respectfully submitted,
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